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Chronic administration of ethyl docosahexaenoate reduces gerbil brain eicosanoid productions following ischemia and reperfusion

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Abstract

Arachidonic acid (AA) and its vasoactive metabolites have been implicated in the pathogenesis of brain damage induced by cerebral ischemia. The membrane AA concentrations can be reduced by changes in dietary fatty acid intake. The purpose of the present study was to investigate the effects of chronic ethyl docosahexaenoate (E-DHA) administration on the generation of eicosanoids of AA metabolism during the period of reperfusion after ischemia in gerbils. Weanling male gerbils were orally pretreated with either E-DHA (100, 200 mg/kg) or vehicle, once a day, for 10 weeks, and subjected to transient forebrain ischemia by bilateral common carotid occlusion for 10 min. E-DHA (200 mg/kg) pretreatment significantly decreased the content of brain lipid AA at the termination of treatment, prevented postischemic impaired regional cerebral blood flow (rCBF) and reduced the levels of brain prostaglandin (PG) $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$, and thromboxane B_2 (TXB_2), as well as leukotriene (LT) LTB_4 and LTC_4 at 30 and 60 min of reperfusion compared with the vehicle, which was well associated with the attenuated cerebral edema in the E-DHA-treated brain after 48 h of reperfusion. These data suggest that the E-DHA (200 mg/kg) pretreatment reduces the postischemic eicosanoid productions, which may be due to its reduction of the brain lipid AA content.

Keywords: Ethyl docosahexaenoate; Arachidonic acid; Eicosanoid productions; Transient forebrain ischemia; Gerbil

1. Introduction

It is well known that cerebral ischemia stimulates the release of arachidonic acid (AA, 20:4n-6) from membrane phospholipids [1,2]. The liberated free AA after ischemia is generally thought to be either reincorporated into the membrane phospholipids [2,3] or the precursor of thromboxanes (TXs), prostaglandins (PGs), leukotrienes (LTs) and other bioactive eicosanoids [4,5], several of which have been implicated in the brain damage following ischemia and reperfusion [6–8]. Arachidonate metabolism is stimulated by ischemia, but the molecular oxygen is required in both cyclooxygenase and lipoxygenase pathways [9]. Presumably, reestablishment of blood flow restores tissue oxygen and permits the conversion of AA to bioactive eicosanoids. Cyclooxygenase enzymes catalyze the formation of unstable cyclic endoperoxides PGG_2 and PGH_2, leading to the generation of prostanoids including vasoactive TXA_2 and prostacyclins [9]. TXA_2 is a potent vasoconstrictor and platelet aggregator that can limit reperfusion following ischemia [10]. Inhibition of cyclooxygenase by indomethacin has been shown to prevent impaired cerebral blood flow (CBF) and brain edema after ischemia [11]. The lipoxygenase pathway of AA metabolism results in the formation of LTs [12]. These LTs, especially the 5-lipoxygenase-derived LTs (LTB_4, LTC_4 and LTD_4), increase vascular permeability [10]. Treatment with a 5-lipoxygenase inhibitor, AA-861, has been found to reduce the increase of brain water content associated with reperfusion after ischemia [13].

Docosahexaenoic acid (DHA, 22:6n-3) is the most commonly encountered n-3 polyunsaturated fatty acid (PUFA) found in various human tissues. This fatty acid is also known as a major n-3 acid rich in fish oils, as well as eicosapentaenoic acid (EPA, 20:5n-3), which may have beneficial effects against several diseases by competing with the PUFA of the n-6 series, in particular AA, for esterification into cellular phospholipids and eicosanoid synthesis, favoring the formation of less biologically active derivatives of three series of prostanoids and five series of

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LTs, respectively [14]. Recently, Umemura et al. [15] showed that dietary DHA produced antithrombotic effects via inhibition of TXB_2 formation in whole blood and caused a reduction in the size of ischemic cerebral lesions in a middle cerebral artery (MCA) thrombosis rat model. Furthermore, Okada et al. [16] reported that chronic administration of DHA contributes to protection against neuronal damage in the hippocampal CA1 region and reduced cognitive deficit caused by transient forebrain ischemia. Our recent study suggested that pretreatment with ethyl docosahexaenoate (E-DHA), the form of esterified DHA that is more effectively absorbed and incorporated into tissues than its free form [17], protected against oxidative brain injury in ischemic gerbils [18]. However, the effects of DHA or E-DHA treatment on the generation of eicosanoids of AA metabolism during the reperfusion after cerebral ischemia were not investigated in these studies.

The present study was therefore conducted to evaluate the effects of chronic administration of E-DHA on the postischemic eicosanoid productions following 10 min of transient cerebral ischemia in the Mongolian gerbil. First, we evaluated the effect of E-DHA on the fatty acid composition of brain lipid at the termination of treatment, and, second, we evaluated the effects of E-DHA on the levels of postischemic brain eicosanoids $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2) and TXB_2 (a stable metabolite of TXA_2), as well as LTB_4 and LTC_4.

2. Materials and methods

2.1. Animals and treatments

Weanling male Mongolian gerbils (21-day-old, obtained from the Experimental Animal Center of Zhejiang Medical University, China) were randomly divided into two groups. One group (E-DHA group) was orally treated with E-DHA emulsified in 5% gum Arabic solution at a dose of 100 or 200 mg/kg (1 ml/kg), once a day, for 10 weeks (the timing of treatment according to Gamoh et al. [19]); the other group (vehicle group) was treated with a similar volume of vehicle alone. Before and after ischemia or reperfusion, gerbils were housed six to a cage at a constant room temperature of 21–22°C under a light/dark cycle of 12/12 h (7:00 a.m./7:00 p.m.). The animals were allowed free access to pelleted food and drinking water. Adaptation and experiments were carried out in accordance with internationally accepted principles and national laws concerning the care and use of laboratory animals, and were approved by the Ethical Committee of the University of Nanjing.

2.2. Fatty acid analysis of brain lipids

Cerebral hemispheres were quickly removed from experimental animals at the termination of treatment, frozen in liquid nitrogen and stored at −80°C until fatty acid analysis. The frozen tissues were weighed and homogenized in a chloroform/methanol (2:1, v/v) mixture for lipid extraction [20]. The solvent mixture was evaporated to a known volume under nitrogen, and the fatty acids were converted to their fatty acid methyl esters by acid methanolysis with BF_3-methanol (Sigma) at 60°C for 1 h [21]. Fatty acid methyl esters were analyzed by a gas–liquid chromatograph (HP 5890; Hewlett-Packard, Avondale, PA) equipped with a flame-ionization detector and a silica capillary column (30 cm×0.32 mm i.d., SP-2330, Supelco, Bellefonte, PA). The oven temperature was programmed to rise from 170°C to 240°C, and the detector temperature was set at 270°C. Identification of the fatty acids was made by comparison of retention times with those of known standards run under the same conditions. Peak areas were calculated with a Hewlett-Packard HP3396 series II integrator, and the fatty acid concentrations were reported as percent of total fatty acid content.

2.3. Surgical preparation

At the termination of treatment, the gerbil (40–60 g) was anesthetized by inhalation of 2% halothane in 30% oxygen/70% nitrous oxide. A midline ventral incision was made in the gerbil's neck, and the trachea was then cannulated with PE-10 polyethylene catheter. A PE-10 polyethylene catheter was inserted into the left femoral artery to monitor the arterial blood pressure and to obtain an arterial blood sample for blood analysis (the removed blood volume was replaced with saline to avoid hypovolemia). Transient forebrain ischemia was produced by clipping both the right and the left common carotid arteries with atraumatic aneurysm clips for 10 min. Blood flow during the reperfusion after removal of the clips was visually confirmed. Rectal and brain temperatures (measured by a rectal probe and a tympanic probe, respectively) were maintained at 37.0±1.0°C during the ischemia and the early postischemia period by placing the animal in a heated box and using a controlled heating lamp [22]. The same surgically operated animals without carotid occlusion were served as sham animals. Halothane anesthesia was turned off immediately after cerebral reperfusion except for the regional CBF (rCBF) measure study.

As an experimental protocol, we set up four treatment groups: (1) sham (sham+vehicle), (2) E-DHA (sham+E-DHA), (3) I/R (ischemia/reperfusion+vehicle) and I/R+E-DHA (ischemia/reperfusion+vehicle+E-DHA). In each treatment group, the animals were divided into four subgroups to examine (1) the physiological parameters; (2) rCBF; (3) eicosanoids $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2, as well as LTB_4 and LTC_4; and (4) cerebral edema. A total of 250 gerbils were used, and the animals were excluded from the study due to death.

2.4. Measurement of rCBF

In animals used for measurement of rCBF, the head was fixed in a stereotaxic frame, and burr holes were made bilaterally over the frontal and parietal cortex. Four Teflon-coated

Table 1
Physiological parameters in vehicle and E-DHA pretreated gerbils

Group	*n*	Core temperature (°C)	MABP (mm Hg)	$PaCO_2$ (mm Hg)	PaO_2 (mm Hg)	pH
30 min pre-ischemia						
Vehicle	6	37.1±0.4	72.8±8.4	36.5±6.4	95.7±6.5	7.37±0.09
E-DHA (100 mg/kg)	6	37.0±0.2	72.8±7.4	35.8±5.8	95.6±7.2	7.37±0.14
E-DHA (200 mg/kg)	6	37.0±0.3	72.8±7.5	35.3±5.2	95.7±2.4	7.37±0.16
30 min after reperfusion						
Vehicle	6	36.9±0.6	67.7±3.9	35.2±4.5	94.9±4.4	7.36±0.11
E-DHA (100 mg/kg)	6	36.8±0.5	66.3±5.7	34.7±5.0	94.7±5.3	7.36±0.27
E-DHA (200 mg/kg)	6	36.8±0.7	66.1±6.8	35.6±7.9	94.8±7.7	7.36±0.18
60 min after reperfusion						
Vehicle	6	37.0±0.8	71.6±8.1	36.1±2.1	95.5±6.3	7.36±0.17
E-DHA (100 mg/kg)	6	37.0±0.4	70.4±6.3	35.6±3.2	95.6±7.1	7.36±0.23
E-DHA (200 mg/kg)	6	37.0±0.1	70.5±7.6	35.2±1.9	95.7±1.1	7.37±0.12

Values are means±S.E.M. No significant difference was found with one-way ANOVA followed by Fisher's protected LSD post hoc tests.

platinum electrodes (125 μm, diameter) were placed stereotactically 0.5 mm with micromanipulators into the cerebral cortex, and the electrodes were fixed in place with acrylic cement. An Ag/AgCl reference electrode was placed subcutaneously in the right leg. Measurements of rCBF were taken prior to bilateral carotid occlusion, during occlusion, and 5, 30 and 60 min after reperfusion via the hydrogen clearance method as described in detail elsewhere [23]. A hydrogen clearance curve was recorded, and the rCBF was calculated as ml/(100 g min).

2.5. Determination of brain eicosanoids

The gerbils were perfused transcardially with saline, and the brains were removed at 30 or 60 min of reperfusion under anesthesia and then immediately frozen with immersion liquid nitrogen. Bilateral hemispheres were weighed and homogenized in cold methanol using a polytron homogenizer (Brinkmann Instruments, Westbury, NY), followed by addition of 0.1 M potassium phosphate buffer (pH 7.4) and rehomogenized. The suspension was centrifuged at 1500×*g* to remove the particulate matter. Supernatants were diluted with an appropriate volume of distilled water to yield a final concentration of 14% methanol, and the pH was adjusted to 3.5–4.0 with 1 M phosphoric acid. Samples were loaded onto reversed phase (C-18) Sep-Pak cartridges (Waters, Bedford, MA), which had been prepared by washing with methanol, followed by a slow percolation with 5 ml 0.5% EDTA (pH 7.4, in 15% aqueous methanol). Samples were washed onto the Sep-Pak cartridges with 5 ml of the 15% aqueous methanol and extracted by passing 5 ml of 30% aqueous methanol, followed by 5 ml methanol, through the cartridges. The 30% methanol and the methanol effluents were pooled and evaporated to dryness in vacuo. The samples were stored frozen until enzyme immunoassay (EIA) or radioimmunoassay (RIA) for eicosanoids was run.

Standards ($PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TXB_2, LTB_4 and LTC_4) and antibodies were purchased from Cayman Chemical (Ann Arbor, MI). $PGF_{2\alpha}$, LTB_4 and LTC_4 were determined by EIA as previously described with slight modifications [24]. Briefly, 50 μl of the samples or appropriate amount of the standard eicosanoid solution was pipetted into a 96-well plate, which had been previously coated with monoclonal mouse antirabbit IgG (2 μg/well). The coated plates were washed three times with a 0.01-M phosphate buffered saline (pH 7.4). To each, 50 μl of acetyl cholinesterase (ACE)-linked eicosanoid conjugate and 50 μl of appropriate special antibody were added; the plates were shaken and incubated overnight at room temperature. The following morning the plates were washed as before. Two hundred microliters of a freshly prepared mixture of 0.5 mM Ellman's reagent [5′ 5-dithiobis (2-nitrobenzoate)] and 0.69 mM of ACE substrate [acetylthiocholine iodide in

Table 2
Fatty acid profiles of brain lipid from gerbils after vehicle and E-DHA pretreatment

Group	*n*	AA (% of total fatty acid)	DHA (% of total fatty acid)
Vehicle	6	11.80±0.12	15.01±0.37
E-DHA (100 mg/kg)	6	11.77±0.88	15.25±0.56
E-DHA (200 mg/kg)	6	9.68±0.74*	17.34±0.39*

Values are means±S.E.M.

* $P<.01$ vs. vehicle with one-way ANOVA followed by Fisher's protected LSD post hoc tests.

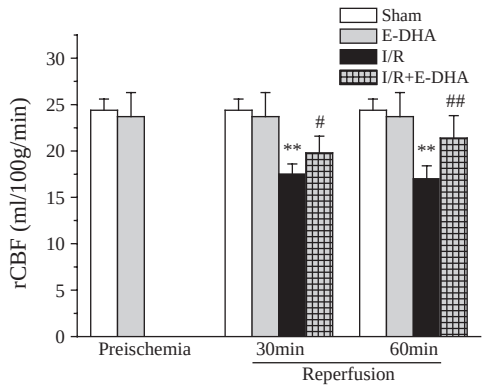

Fig. 1. Effects of pretreatment (10 weeks) with oral administration of vehicle or E-DHA [200 mg/(kg day)] on the rCBF in gerbils prior to ischemia and the 30- and 60-min postischemic reperfusion. Each column represents the mean±S.E.M. of six animals. $^{**}P<.01$, I/R vs. sham; $^{\#}P<.05$, $^{\#\#}P<.01$, I/R+E-DHA vs. I/R. Statistical analysis was performed by unpaired Student' *t* test.

0.01 M potassium phosphate butter (pH 7.4)] were added to each well, and the plates were shaken in the dark for 1–18 h until adequate color was developed. The absorbance at 412 nm was determined using a model 550 microtiter plate reader (Bio-Rad, USA). 6-Keto-$PGF_{1\alpha}$ and TXB_2 were determined using commercially available RIA kits from New England Nuclear (Boston, MA). Assays were performed strictly according to the manufacturer's instructions. The values were expressed as nanograms per gram weight of brain.

2.6. Evaluation of cerebral edema

Cerebral edema was evaluated through measurement of brain water content after 48 h of reperfusion, using wet weight–dry weight ratios [25]. Freshly dissected hemispheres were weighed, dried at 105°C for 24 h and then reweighed. The percentage of water (H_2O%) was calculated as 100×(wet weight−dry weight)/wet weight.

2.7. Statistical analysis

All data were reported as mean±S.E.M. Data analysis was performed using one-way analysis of variance (ANOVA) followed by Fisher's protected LSD post hoc tests or unpaired Student's t test for multiple comparisons. The level of difference was considered significant at $P<.05$.

3. Results

Animals on both the vehicle and E-DHA (100 or 200 mg/kg) dietary administrations appeared healthy and grew well. There were no significant differences in food intake, body

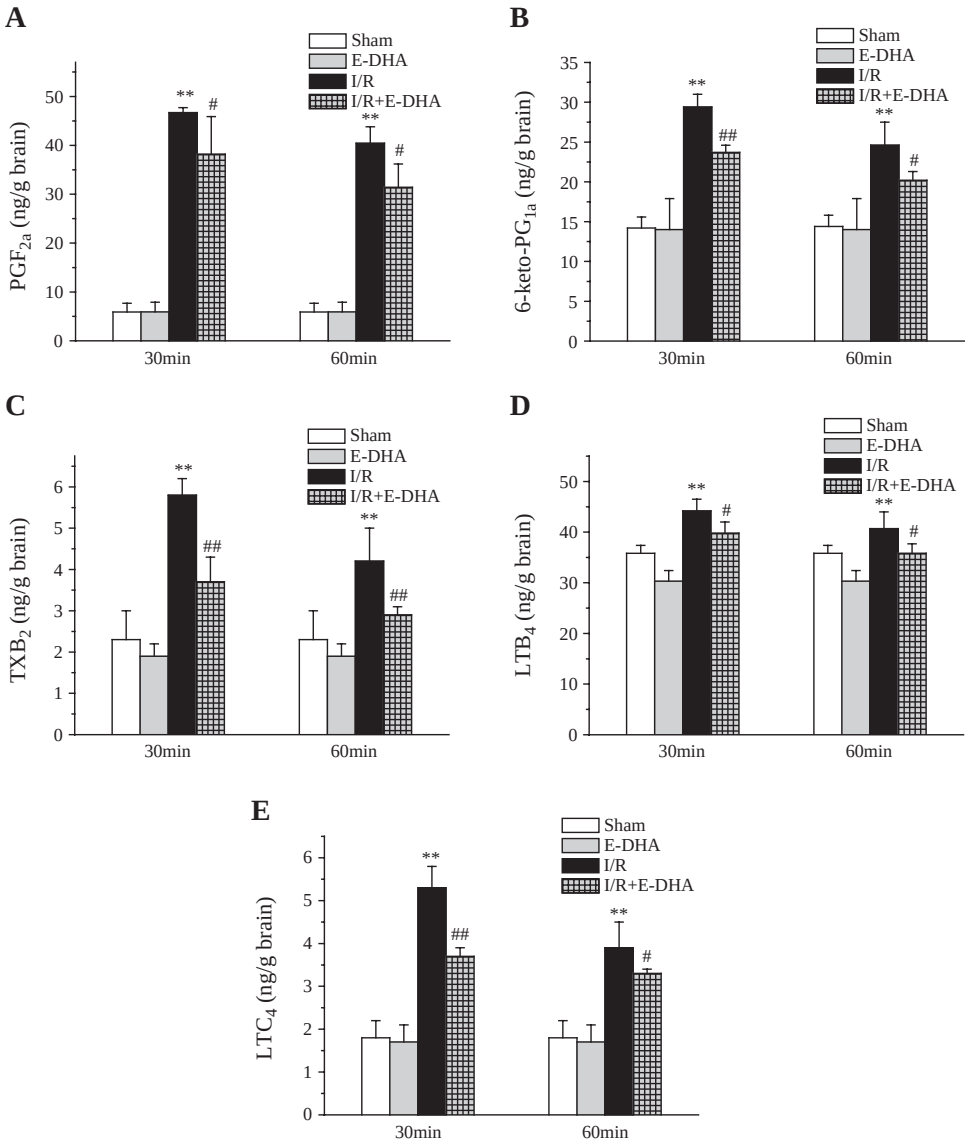

Fig. 2. Effects of pretreatment (10 weeks) with oral administration of vehicle or E-DHA [200 mg/(kg day)] on the brain eicosanoid productions in gerbils at the 30- and 60-min postischemic reperfusion. (A) Brain $PGF_{1\alpha}$ level; (B) brain 6-keto-$PG_{1\alpha}$ level; (C) brain TXB_2 level; (D) brain LTB_4 level; (E) brain LTC_4 level. Each column represents the mean±S.E.M. of six animals. $^{**}P<.01$, I/R vs. sham; $^{\#}P<.05$, $^{\#\#}P<.01$, I/R+E-DHA vs. I/R. Statistical analysis was performed by unpaired Student' t test.

weight and body temperature between animals receiving E-DHA administration over a 10-week period and those on vehicle diet (data not shown). The mean arterial blood pressure (MABP), arterial blood gases and core temperature were similar in both vehicle-treated and E-DHA-treated animals as shown in Table 1.

3.1. Brain AA and DHA

A dose-dependent effect of chronic administration of E-DHA on brain fatty acid composition is shown in Table 2. Chronic administration of 200 mg/(kg day) E-DHA over 10 weeks significantly decreased the AA content in the brain ($P<.01$) and simultaneously resulted in marked increase in brain DHA value ($P<.01$), but did not cause significant changes in the contents of total fatty acids when compared with those in the vehicle-treated animals. However, chronic administration of 100 mg/(kg day) E-DHA changed neither the content of AA ($P>.05$) nor the DHA content ($P>.05$) in the brain.

3.2. Regional CBF

Fig. 1 shows the rCBF in the vehicle- and E-DHA (200 mg/kg)-pretreated gerbils. No significant differences ($P>.05$) in the pre-ischemic rCBF were found between the sham [24.4±1.2 ml/(100 g min)] and the E-DHA [23.7±2.6 ml/(100 g min)] groups. During bilateral carotid artery occlusion, the rCBF decreased to less than 5 ml/(100 g min) in both the I/R and I/R+E-DHA groups. Five minutes after reperfusion, a remarked hyperemia was noted; flow increased to 43.1±4.2 ml/(100 g min) in the I/R gerbils and to 41.2±3.8 ml/(100 g min) in the I/R+E-DHA gerbils. However, I/R+E-DHA gerbils sustained higher rCBF than the I/R animals at 30 min [19.8±1.8 vs. 17.5±1.1 ml/(100 g min), $P<.05$] and 60 min [21.4±2.4 vs. 17.0±1.4 ml/(100 g min), $P<.01$] of reperfusion.

3.3. Brain eicosanoids

There were no significant differences ($P>.05$) in the levels of brain eicosanoids between the sham and E-DHA (200 mg/kg) groups. However, as shown in Fig. 2, 10 min of ischemia followed by different periods of reperfusion resulted in significant increases in brain prostanoid and LT productions in the I/R gerbils: prostanoid $PGF_{2\alpha}$ increased from 5.9±1.8 (sham) to 46.7±1.0 and 40.4±3.4 ng/g brain at 30 and 60 min of reperfusion, respectively (Fig. 2A); 6-keto-$PGF_{1\alpha}$ increased from 14.1±1.4 (sham) to 29.4±1.6 and 24.6±2.9 ng/g brain at 30 and 60 min of reperfusion, respectively (Fig. 2B); TXB_2 increased from 2.3±0.7 (sham) to 5.8±0.4 and 4.2±0.8 ng/g brain at 30 and 60 min of reperfusion, respectively (Fig. 2C). The leukotriene LTB_4 increased from 35.8±4.1 (sham) to 44.2±2.3 and 40.7±3.3 ng/g brain at 30 and 60 min of reperfusion, respectively, (Fig. 2D); LTC_4 increased from 1.8±0.4 (sham) to 5.3±0.5 and 3.9±0.6 ng/g brain at 30 and 60 min of reperfusion, respectively (Fig. 2E).

The I/R+E-DHA groups had lower levels of all the three prostanoids and two LTs at the examined time point of postreperfusion than the I/R groups, as follows (in ng/g brain): $PGF_{2\alpha}$ 38.2±7.7 at 30 min ($P<.05$) and 31.4±4.8 at 60 min ($P<.05$); 6-keto-$PGF_{1\alpha}$ 23.7±0.9 at 30 min ($P<.01$) and 20.2±1.1 at 60 min ($P<.05$); TXB_2 3.7±0.6 at 30 min ($P<.01$) and 2.9±0.2 at 60 min ($P<.01$); LTB_4 39.8±2.2 at 30 min ($P<.05$) and 35.8±1.9 at 60 min ($P<.05$); LTC_4 3.7±0.2 at 30 min ($P<.01$) and 3.3±0.1 at 60 min ($P<.05$). The E-DHA was shown to significantly reduce the levels of postischemic brain eicosanoids as compared with the vehicle.

3.4. Cerebral edema

There was no significant difference ($P>.05$) found in the brain water content, an index of cerebral edema, between the sham group (78.38±0.67%) and the E-DHA (200 mg/kg) group (78.34±0.61%) (Fig. 3). The brain water content, observed after 48 h of reperfusion, was significantly increased ($P<.01$) in the I/R group (83.41±0.55%) as compared to the sham group, but was decreased significantly in the I/R+E-DHA group (81.29±0.57%) compared with the I/R group, which indicated that E-DHA pretreatment produced a significant reduction in postischemic cerebral edema.

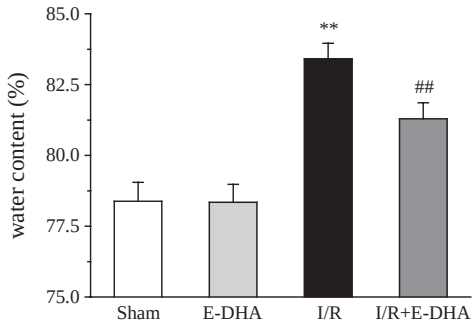

Fig. 3. Effects of pretreatment (10 weeks) with oral administration of vehicle or E-DHA (200 mg/kg day) on the cerebral edema in gerbils at the 48 h of postischemic reperfusion. Each column represents the mean±S.E.M. of six animals. **$P<.01$ I/R vs. Sham, $^{\#\#}P<.01$ I/R+E-DHA vs. I/R. Statistical analysis was performed by unpaired Student's t test.

4. Discussion

The present study clearly shows that chronic administration of E-DHA [200 mg/(kg day)] over 10 weeks significantly decreased the level of brain lipid AA, which markedly reduces the brain eicosanoid productions during the reperfusion which prevents the postischemic rCBF from declining and attenuates the postischemic cerebral edema following 10 min of transient forebrain ischemia in the Mongolian male gerbils.

Arachidonic acid comprises the major portion of free fatty acids, which are liberated from the membrane phospholipids mainly by the action of phospholipidase A_2 (PLA_2) following

cerebral ischemia [1,26]. An increase in the concentrations of AA causes an impairment of the integrity of the membrane and a disturbance of the mitochondrial respiratory chain [27,28], and induces brain edema [29]. At the same time, it has been suggested that AA metabolite eicosanoids are involved in the pathophysiologic consequences of brain ischemia through regulation of cerebral blood flow (CBF), vascular permeability and modulation of neurotransmission [30]. The availability of AA is a rate-limiting factor for the synthesis of eicosanoids in the brain. Under physiological conditions, PLA_2 liberates AA from membrane phospholipids at a rate less than or equal to the rate of free AA reincorporation into the membranes. Thus, a balance between the liberation of AA from and its reacylation into membrane phospholipids results in low levels of free AA [3,31]. However, under conditions of ischemia, the free AA production exceeds utilization, and accumulation ensues [3]. The rapid free AA increase during the ischemia is generally considered to be due to impaired phospholipid reacylation secondary to energy failure/ATP depletion, because the reacylation process requires energy [3,32]. Simultaneously, ischemia-induced glutamate release contributes to the activation of PLA_2, which results in further phospholipid degradation and AA liberation [32,33].

In the absence of selective inhibitor of AA liberation, a potential means of investigating the involvement of AA in ischemic brain is by modification of dietary fatty acid composition. It has been shown that supplementation with n-3 fatty acids, such as DHA and EPA, can alter the membrane fatty acid composition of the brain as these fatty acids compete with AA for incorporation into cell membrane [14], and the localized changes in membrane fatty acid composition may be of critical importance in determining neuronal function and responses to insults [34]. Numerous studies have investigated how dietary n-3 fatty acids may be able to ameliorate some of the deleterious symptoms associated with ischemia/reperfusion [21]. Okada et al. [16] reported that chronic administration of DHA contributes to protection against neuronal damage in the hippocampal CA1 region and reduced cognitive deficit caused by transient forebrain ischemia. They suggested that administration of DHA decreased the brain AA content, which might be attributed to the protective effect of DHA treatment on neuronal damage. The present study agrees with the previous findings and shows that chronic E-DHA (200 mg/kg) administration results in a decrease in brain lipid AA content in the gerbils.

It is rational to speculate that limiting the amount of brain AA, through manipulation by pretreatment with DHA or E-DHA, would reduce the amount of free AA generated during ischemia and, consequently, result in less availability of AA for metabolism into various eicosanoids during the reperfusion. The liberation of AA during the ischemia may be monitored by measuring the metabolites, and the increased metabolite synthesis, as evidenced by high tissue levels of eicosanoids, probably indicates an increased availability of substrate (AA), derived from the breakdown of the membrane. Arachidonic acid is rapidly metabolized by cyclooxygenase with molecular oxygen (e.g., reperfusion) to the cyclic endoperoxides prostaglandin G_2 (PGG_2) or PGH_2 and to prostanoids (TXs and PGs) and by 5-lipoxygenase to hydroxy fatty acids and LTs [6,7]. In this study, we measured the brain eicosanoids $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2, as well as LTB_4 and LTC_4, and found that all the eicosanoids showed parallel significant increases at 30 and 60 min of reperfusion after 10 min of transient cerebral ischemia compared to baseline levels in the vehicle-treated animals. But these eicosanoid levels were all significantly decreased by pretreatment with E-DHA (200 mg/kg) at each observed time point, which suggests that the E-DHA pretreatment could reduce the availability of AA for metabolism. In addition, it was found that enrichment of DHA in lipids attenuated PLA_2 activity [35,36], although the exact mechanism was not known, consequently reducing the levels of AA liberated, a rate-limiting step in the synthesis of biologically active eicosanoids. In our experiments, chronic administration of E-DHA significantly increased the brain lipid DHA content in gerbils, which may also decrease the AA liberation by reducing the PLA_2 activity during the ischemia and contributes to the reduced eicosanoid productions observed in the E-DHA-treated brain during the different periods of reperfusion.

The accumulation of AA metabolites that accompanies the onset of reperfusion may contribute to changes in CBF and the pathophysiology of postischemic cerebral edema and reperfusion injury, particularly in regard to secondary microvascular dysfunction, since some of the metabolites are vasoactive [10]. In general, TXA_2, which is hydrolyzed spontaneously to the stable TXB_2, is the most potent endogenously synthesized vasoconstrictor eicosanoid and a stimulant of platelet aggregation [6,7] and, thus, may act to compromise brain microvascular blood flow. Similarly, $PGF_{2\alpha}$ is a vasoconstrictor that may reduce cerebral perfusion. However, PGI_2 (spontaneously hydrolyzed to the stable 6-keto-$PGF_{1\alpha}$) is a potent vasodilator and the most potent endogenous inhibitor of platelet aggregation. Protective functions have been attributed to the PGI_2 [27]. In regard to the 5-lipoxygenase-derived eicosanoids, LTC_4 and LTD_4 stimulate contraction of smooth muscle and alter vascular permeability. LTB_4 also alters vascular permeability and has a potent chemotactic effect on polymorphonuclear leukocytes. These LTs may modify cerebrovascular tone and play an important role in cerebral edema formation [10]. In the present study, the reduction in LTB_4 and LTC_4 may be attributed to the E-DHA attenuating the cerebral edema after reperfusion, and the decrease of TXB_2 and $PGF_{2\alpha}$ may partially explain the E-DHA preventing the postischemic impaired rCBF (30 and 60 min of reperfusion). Although E-DHA pretreatment also reduced the PGI_2 production, fostering TXA_2 and PGI_2 imbalance that could contribute to postischemic hypoperfusion, formation of platelet microthrombi and impairment of cerebral microcir-

culation [10], as well as EPA, DHA can be expected to produce PGI_3, which has the same action as PGI_2. In addition, the existence of an enzyme system that converts DHA to EPA has been reported [37]. Eicosapentaenoic acid is a relatively poor substrate for cyclooxygenases and is usually converted to eicosanoids including TXA_3 with lower proaggregatory and vasoconstrictive activities than TXA_2 [38]. The remarked hyperemia during the first 5 min of reperfusion in both treated groups may be due to the continued hydrolysis of membrane lipids which results in further rises in certain free fatty acids [39].

The platelets and activated blood components are critical for the production of increased eicosanoids after ischemia [9]. Although we did not measure the platelet aggregation in the present study, Umemura et al. [15] have demonstrated that dietary DHA reduced platelet aggregability and enhanced thrombolytic efficacy of recombinant tissue-type plasminogen activator (rt-PA) in the rat MCA thrombosis model. In addition, DHA esterified in the membrane phospholipids is released in brain ischemia and can be converted to potent lipid mediators as is the case with AA [40]. Marcheselli et al. [40] and Serhan [41] suggested that endogenous DHA was converted in vivo to a 17S series of resolvins (RvD1–RvD6) as well as 10,17S-docosatriene (DT). The novel DHA-derived DT is a potent inhibitor of ischemia-reperfusion-induced polymorphonuclear neutrophil (PMN) infiltration and pro-inflammatory gene induction, and limits stroke brain injury. With microglial cells that liberate cytokines in the brain, the RvDs block tumor necrosis factor-α (TNF-α)-induced interleukin-1β transcripts and are potent regulators of PMN infiltration in vivo. The postischemic inflammatory process is characterized by infiltration of acute inflammatory cells (PMNs) and release of inflammatory mediators. Such inflammatory mediators, including AA and its metabolites (eicosanoids), may play an important role in the pathogenesis of brain edema after cerebral ischemia. In this study, pretreatment with E-DHA (200 mg/kg) significantly increased the brain lipid DHA content, which may contribute to the more free DHA available on activation to produce 17S-containing DT and RvDs, and consequently attenuated brain edema after ischemia and reperfusion. The E-DHA pretreatment may lead to the conversion of fatty acid composition from a predominantly pro-inflammatory AA to an anti-inflammatory DHA profile.

The animals pretreated with E-DHA (200 mg/kg) exhibited no effects on food intake, body weight, body temperature and physiological parameters. Therefore, it is reasonable to assume that the observed differences in postischemic eicosanoid productions in this study resulted from the E-DHA pretreatment. Additionally, we recently demonstrated that the chronic E-DHA administration protects against brain injury through its inhibition of oxygen free-radical formation and lipid peroxidation in ischemic gerbils [18]. Oxygen radicals and lipid peroxidation have been shown to tightly regulate the cycooxygenase and/or 5-lipoxygenase activities, and stimulate the eicosanoid synthesis [42,43]. Thus, the contribution of this possible action of E-DHA to reducing the postischemic eicosanoid overproductions cannot be ruled out in the present study.

In summary, chronic administration of E-DHA (200 mg/kg) exhibits inhibitory effects on the postischemic eicosanoid productions in ischemic gerbils. The effects are most probably due to the reduction of brain lipid AA contents by the E-DHA pretreatment. However, it is still unclear whether the chronic administration of E-DHA could decrease the liberation of AA and the availability of AA for metabolism during the ischemia and reperfusion. Further investigation will be required.

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